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DEVELOPMENT AND GROWTH OF POTATO TUBERS IN MICROGRAVITY

M. E. Cook*, J. L. Croxdale*, T. W. Tibbitts**, G. Goins***, C. S. Brown*** and R. M. Wheeler†

**Botany Department, University of Wisconsin, Madison, WI 53706, U.S.A.*
***Horticulture Department, University of Wisconsin, Madison, WI 53706, U.S.A.*
****Dynamac Corporation, Kennedy Space Center, FL 32899, U.S.A.*
†Biomedical Office, National Aeronautical and Space Administration, Kennedy Space Center, FL 32899, U.S.A.

ABSTRACT

A potato explant consisting of a leaf, its axillary bud, and a small segment of stem will develop a tuber in 10-14 days when grown on earth. The tubers develop from the axillary buds and accumulate starch derived from sugars produced through photosynthesis and/or mobilized from leaf tissue. Potato explants were harvested and maintained in the Astroculture™ unit, a plant growth chamber designed for spaceflight. The unit provides an environment with controlled temperature, humidity, CO_2 level, light intensity, and a nutrient delivery system. The hardware was loaded onto the space shuttle Columbia 24 hours prior to the launch of the STS-73 mission. Explant leaf tissue appeared turgid and green for the first 11 days of flight, but then became chlorotic and eventually necrotic by the end of the mission. The same events occurred to ground control explants with approximately the same timing. At the end of the 16-day mission, tubers were present on each explant. The size and shape of the space-grown tubers were similar to the ground-control tubers. The arrangement of cells in the tuber interior and at the exterior in the periderm was similar in both environments. Starch and protein were present in the tubers grown in space and on the ground. The range in starch grain size was similar in tubers from both environments, but the distribution of grains into size classes differed somewhat, with the space-grown tubers having more small grains than the ground control tubers. Proteinaceous crystals were found in tubers formed in each condition.

INTRODUCTION

The ability of potatoes to form sessile tubers from axillary buds has been known for many years (Vöchting 1887), and this phenomonon occurs even with excised leaves and their axillary buds, i.e. explants (Gregory, 1965; Duncan and Ewing, 1984). As the tuber develops on the explant, photosynthesis, transport, and starch synthesis must take place. The leaves photosynthesize, some of the resulting assimilate is transported as sucrose and converted to starch in the expanding tuber. These explants provide a model system for studying tuber formation because a substantial tuber develops in a relatively short time period from an inconspicuous lateral bud (Wheeler and Tibbitts, 1985). Both cell division as well as cell expansion take place during tuber development (Cutter, 1992). Single node explants potentially are useful in spaceflight because they provide a compact model system to study development, photosynthesis and metabolism in plants. These processes are critical in plant-based bioregenerative life support systems that are designed to provide food and O_2, scrub CO_2 from the cabin air, and provide potable water from gray water for long-term space exploration and habituation. The objective of this experiment was to determine whether tubers formed during spaceflight would be similar to those grown on earth and potentially serve as part of the life support system for crew members on extended space missions.

MATERIALS AND METHODS

Potato Explant System

Solanum tuberosum (cv. Norland) mother plants, grown from *in vitro* propagated plantlets, were started 6 weeks before the scheduled launch. Successive plantings were started at 2-week intervals to provide additional explants for launch delays. All plants were grown at 21°C, 80% RH, 150 μmol m^{-2} s^{-1} PPFD for a 12-h photoperiod and approximately 350 μol mol^{-1} CO_2 in a walk-in growth chamber at the Kennedy Space Center. Flight explants were harvested from the 7th-8th leaves of the mother plants, counting basipetally from the youngest leaf that is 1 cm in length. Each explant, consisting of a leaf, axillary bud, and small section of stem, was trimmed of excess laminar tissue and then planted into a tray filled with moistened arcillite, calcined clay particles. Light-tight flexible gaskets were placed around the petiole base of each explant and opaque acrylic plates were secured over the base of the explants and arcillite for their containment during spaceflight.

The above protocol was used in baseline studies performed at Madison. Mother plants were grown in the Biotron of the University of Wisconsin-Madison, harvested, and trimmed according to the same procedures used at Kennedy Space Center. When the ground control experiment was performed, the Astroculture™ growth unit was placed in a controlled environment room at the Biotron with the temperature, relative humidity, and CO_2 level approximating the conditions of the space shuttle middeck during the flight.

Astroculture™ Growth Unit

The Astroculture™ unit, which was developed at the Wisconsin Center for Space and Automation Robotics (WCSAR), provides a controlled environment for growing plants in microgravity. The flight package was programmed to maintain the plant chamber at 21°C, 80% RH, and a minimum of 500 ppm CO_2. Above the plant chamber was an array of red and blue LEDs to provide 150 μmol m^{-2} s^{-1} of PPFD during the 12 h light period. Water was delivered to the arcillite-containing plant tray by a negative pressure system (Duffie *et al.*, 1995). Following the loading of the potato explants into the tray, the tray was inserted into the unit and maintained under auxillary power until installation into the middeck locker and launch of the Space Shuttle.

Data were recorded every 10 min on each environmental parameter in the growth unit and stored in an internal computer for post-flight retrieval. These data also were transferred on a daily basis to a shuttle computer for downlinking to the ground during the mission. A video camera located at the top of the plant chamber allowed visual monitoring of the explants during the mission.

Microscopic Procedures

Prior to flight, baseline studies were conducted on ground control explants of several ages by light microscopy (LM) and scanning electron microscopy (SEM). Hand sections of leaf, petiole, stem and tuber tissue were examined by bright-field and fluorescence microscopy, using sections stained with I-KI and Commasie Blue. Sections were examined with a Zeiss Axioplan and photographed using Kodak 2415 or Ektachrome 400 film. Tuber starch grains were collected onto cover slips by abrading the cut surface of a tuber with a knife blade. Cover slips were attached to SEM stubs with glue and coated with 10nm of gold-palladium and examined at 10 kV with an Hitachi Model S-570. Images were recorded on Polaroid PN 55 film. Using an image analysis program (Cue 2, Olympus Corp.), starch grain length (longest axis) was determined from SEM photographs of whole granules collected from space and ground control tubers. More than 800 measurements were made for tubers formed

in each environment.

RESULTS AND DISCUSSION

Images downlinked from space indicated that the explants were healthy early in the flight; similar images were obtained for ground control explants grown in the flight unit at Madison following the mission. The leaves were fully turgid, dark green, and epidermal hairs were visible on their surfaces. CO_2 was maintained near 500 μmol mol^{-1} in the light by additions of CO_2 from a reservoir, and rose during the dark cycle as a result of tissue respiration. A similar pattern was apparent for ground control explants. On day 11 the video images of flight explants indicated that leaf tissue was becoming chlorotic and the daily fluctuations in CO_2 level began to dampen. Demand for water also declined at this time. By the end of the mission, explant leaf tissue was necrotic. The ground control explants began to senescence one day later than spaceflight explants, but with a similar pattern of chlorosis followed by necrosis of the leaf and rachis.

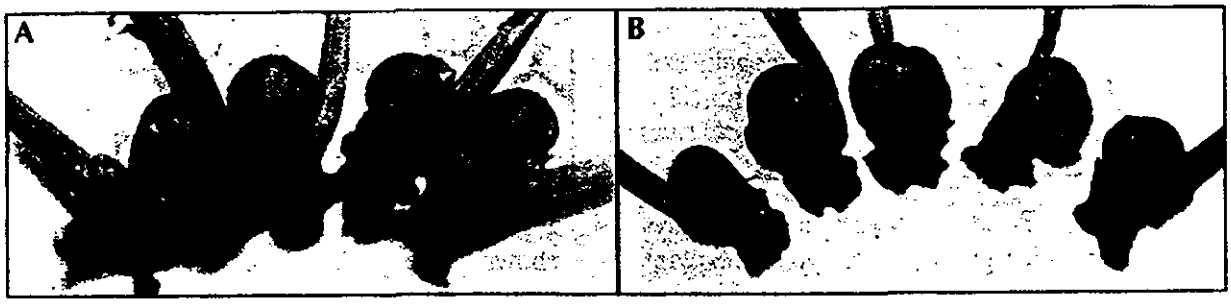

Fig. 1AB. Potato tubers produced by the explant system after 16-days growth in microgravity (A) and on earth (B).

Upon return to earth, the explants were removed from the Astroculture™ unit and photographed. The size and shape of the space-formed tubers were similar to ground control tubers (Figure 1AB). Periderm color was similar between space and ground tubers. All explants showed signs of senescence in the chlorosis and/or necrosis of laminar tissue and the death of the leaf rachis basipetally, but had turgid petioles and stems (Figure 2AB). Senescence of leaf and rachis tissue of the space-flight and ground control explants was advanced compared to explants in the baseline studies (data not shown). However, the space-grown explant that formed the smallest tuber had green leaf tissue in the terminal leaflet and one of the first pair of leaflets (Figure 2B). The entire rachis and petiole of this explant were turgid and dark green in contrast to the other four explants (cf. Figure 2B to 2A). Although the capacity to form a tuber is under developmental control (Ewing, 1985), tubers produced from explants of similar developmental age varied. Although the explants in Figure 2AB were harvested as the 7th leaves from their respective mother plants, based on total leaf length, the explant in Figure 2A was developmentally younger than the explant in Figure 2B; explant lengths were 26.5 cm and 27.2 cm, respectively, prior to trimming.

The arrangement of tuber cells is highly ordered and differs by region (Figure 3)(Cutter, 1992). Located at the tuber periphery is the periderm, which develops from the epidermis of the axillary bud during tuber formation. The tissue consists of multiple layers of cells which are stacked directly on top of one another from layer to layer. Cells in the tuber center are packed in a honeycomb configuration, with those of smaller diameter closer to the periderm. No change either in the number of periderm layers or the overall geometric configuration of cells was detected in space-grown tubers (Fig. 4). Decisions regarding the planes of cell divisions that yield the cellular pattern

in the tuber may have taken place while the leaves were on the mother plant and under the influence of gravity.

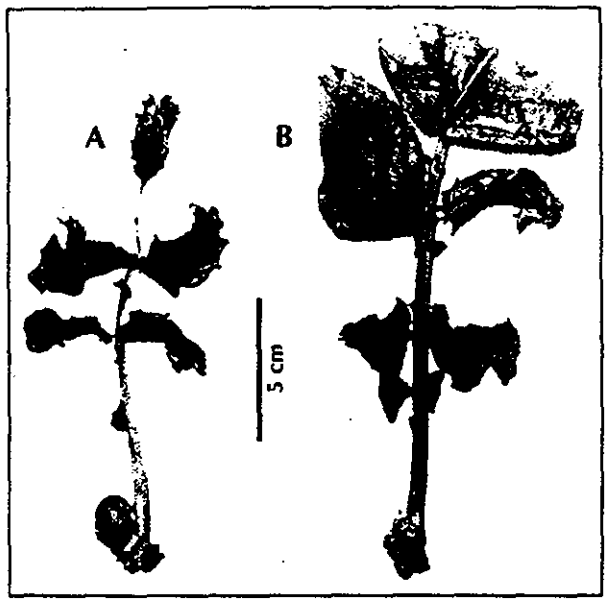

Fig. 2AB. Potato explants after 16-days growth in microgravity. A typical explant showing chlorosis and decay of the leaf lamina and upper leaf rachis, but with a well-developed sessile tuber (A). The explant producing the smallest tuber had green leaf tissue at the terminus, a turgid rachis and petiole (B). Both explants were taken from the 7th leaves from their mother plants; explant length prior to trimming was 26.5 and 27.2 cm for A and B, respectively.

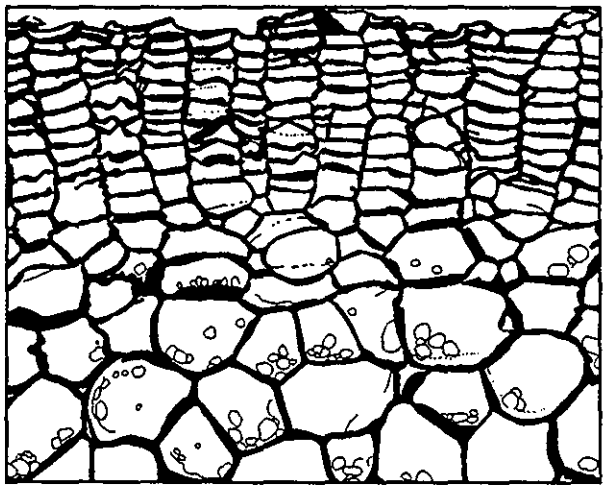

Fig. 3. Diagram of potato tuber transection showing the periderm, a multilayered tissue with cells stacked directly on top of one another from layer to layer, and interior cells in a honeycomb arrangement. Redrawn from Cutter (1992).

The distribution of starch in cells across the tuber appeared homogeneous in the interior cells, and no starch was present in the periderm cells (Figure 4), a pattern also seen in ground control tubers. Starch grains of ground control and space-grown tubers that were fixed following return to earth were displaced toward one side of the cell, in agreement with previous reports (Gray and Edwards, 1968; Grif *et al.*, 1988; Moore *et al.*, 1986, 1987; Volkmann *et al.*, 1986). Large proteinaceous crystals were located interior to the periderm in the outer regions of tubers formed in gravity and microgravity conditions (Figure 4).

The starch grains from spaceflight tubers were similar in shape and size range to those of ground control tubers (Figure 5AB) and similar to ground-grown tubers in baseline studies (data not shown). The smaller grains were spherical, while the larger grains were eccentric; all grains had a smooth surface. Analysis of the longest granule axis showed that the size range was similar in both environments. The size of Norland starch granules from tubers formed in baseline studies ranged from 2 to 40 μm in the longest axis, close to ranges reported in other potato cultivars (Geddes *et al.*, 1965). Although the percentage of each sample distributed among size classes ranging from < 5μm to between 20.1 to 35 μm was similar, the starch grains in the smaller size categories were slightly more numerous in spaceflight tubers than in ground control tubers (Figure 6), while the smallest (<5

Fig. 4. Organization of tuber tissue formed in microgravity. The periderm is present as a series of layers in which cells are stacked on top of cells. The cells internal to the periderm contain starch and proteinaceous crystals (dark objects).

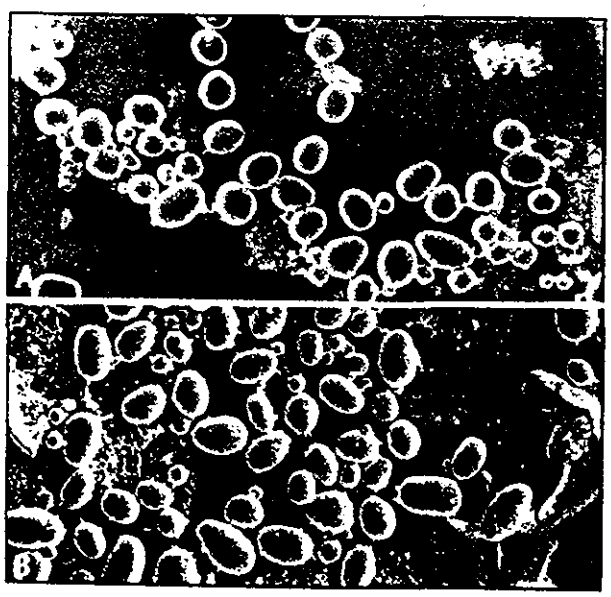

Fig. 5AB. Starch grains from tubers formed in microgravity (A) and on earth (B).

μm) and moderately large grains (10.1 to 15 μm) were somewhat more numerous in the ground control sample. The tendency for more numerous, smaller grain sizes may indicate that development of space tubers lags that of ground controls because small grain size is associated with younger tissue (Badenhuizen, 1968; French, 1984). Alternatively, there are reports (Volkmann and Sievers, 1990; Moore *et al.*, 1986; Moore, 1990) indicating that the number and/or volume of starch grains in roots is reduced as the time spent in space increases.

Animal embryogenesis is dramatically affected when fertilization and subsequent development take place under the influence of microgravity (Classen and Spooner, 1994), but plant morphology is seldom changed because preformed structures are already present in the seed or plant sent aloft. Plants are capable of producing fertile seed in space from plants grown through one complete reproductive cycle, although plants are often shorter, produce fewer leaves and have smaller fruits and seeds. Although spaceflight reportedly affects differentiation, the body of results on plants indicates no consistent effect (Halstead and Dutcher, 1987; Classen and Spooner, 1994). Differentiation of plant tissue in spaceflight is reportedly more rapid during somatic embryogenesis (Theimer *et al.*, 1986) and seedling development (Brown *et al.*, 1992), but growth is reduced in root cells which differentiate prematurely (Halstead and Dutcher, 1987). In the microgravity environ-

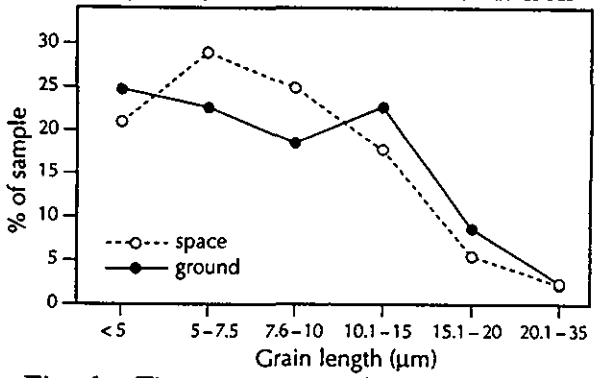

Fig. 6. The percentage of the starch grain sample by size category formed in microgravity and on earth. N=>800 for each sample.

ment, pea internodes are markedly elongated (Merkys *et al.*, 1975) and the number of lateral roots in lettuce and cress is increased (Merkys and Laurinavičius, 1990), but root tip growth and regeneration of the root cap are inhibited (Moore *et al.*, 1987). Further alterations in plant growth (Merkys *et al.*, 1983; Merkys and Laurinavičius, 1990, Cowles *et al.*, 1984), meristematic activity (Krikorian and O'Connor, 1982, Merkys and Laurinavičius, 1990; Grif *et al.*, 1988; Darbelly *et al.*, 1989; Levine and Krikorian, 1992) and cell structure (Cowles *et al.*, 1984; Kuang *et al.*, 1996) have been reported.

A constellation of structural changes are associated with the differentiation of a potato tuber from an axillary bud. Potato tubers formed in the explant system under microgravity have the same gross morphology, the same configuration of cells and tissues, and the same sizes, shapes and surface character of starch granules as tubers formed in a 1 *g* environment. Spaceflight does not alter these fundamental features of tuber differentiation.

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